Amendments to the Specification:

Please amend the title on page 1 as follows:

PHARMACEUTICAL AGENTS [[DRUG]]

Please add the following paragraph to page 1 after the title:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a National Stage application under 35 U.S.C. §371 and claims benefit under 35 U.S.C. §119(a) of International Application No. PCT/JP04/004917 having an International Filing Date of April 5, 2004, which claims the benefit of priority of U.S. Application No. 60/459,644 having a filing date of April 3, 2003.

Please amend the paragraph bridging pages 1 and 2 as follows:

Pim-1 is a serine/threonine kinase initially identified in T cell lymphomas caused by murine leukemia virus (MuLV) as a gene frequently activated by leukemia virus insertion (Cuypers, H.T., Selten, G., Quint, W., Zijlstra, M., Maandag, E.R., Boelens, W., van Wezenbeek, P., Melief, C., Berns, A. "Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region." Cell, 37:141-150, 1984; and Selten, G., Cuypers, H.T. & Berns, A. "Proviral activation of the putative oncogene Pim-1 in MuLV induced T-cell lymphomas." EMBO J, 4:1793-1798, 1985 ~~Comerford, K.M., Wallace, T.J., Karhausen, J., Louis N.A., Montalto, M.C., Colgan, S.P. "Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene." Cancer Res., 62:3387-3394, 2001; and Niizeki, H., Kobayashi, M., Horiuchi, I., Akakura, N., Chen, J., Wang, J., Hamada, J., Seth, P., Katoh, H., Watanabe, H., Raz, A., Hosokawa, M. "Hypoxia enhances the expression of autocrine motility factor and the motility of human pancreatic cancer cells." Br. J Cancer, 86:1914-1919, 2002~~). Further, Pim-1 in the cytoplasm has been reported to function as a factor for inhibiting apoptosis in various hematopoietic cells (Pircher, T.J., *et al.* "Pim-1 kinase protects hematopoietic FDC cells from genotoxin-induced death." Oncogene, 19:3684-3692, 2000; and Lilly, M. & Kraft, A. "Enforced expression of the Mr 33,000 Pim-1 kinase enhances factor-independent survival and inhibits apoptosis in murine myeloid cells." Cancer Res., 57:5348-

5355, 1997 ~~Cuypers, H.T., Selten, G., Quint, W., Zijlstra, M., Maandag, E.R., Boelens, W., van Wezenbeek, P., Melief, C., Berns, A. "Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region." Cell, 37:141-150, 1984; and Selten, G., Cuypers, H.T. & Berns, A. "Proviral activation of the putative oncogene Pim-1 in MuLV induced T-cell lymphomas." EMBO J, 4:1793-1798, 1985~~). Therefore, substances that can inactivate Pim-1 would be effective for preventing/treating solid cancers, and various Pim-1-induced disorders.

Please amend the Brief Description of the Drawings on page 5, lines 5-8 as follows:

Fig. 6 shows the results of Western blotting to detect Pim-1 in three cell lines ~~detecting Pim-1 by Western blotting~~.

Fig. 7 shows the results of Western blotting to detect Pim-1 in the cells treated with a calpain inhibitor ~~detecting Pim-1 by Western blotting~~.

Fig. 8 shows the results of protein electrophoresis of ubiquitinated Pim-1 ~~performing protein electrophoresis using transformed cells~~.

Fig. 9 shows the results of dominant negative Pim-1 expression in transformed cells ~~performing protein electrophoresis using transformed cells~~.

Please amend the paragraph on page 5, lines 16-26 as follows:

In the present invention, "serine/threonine kinase Pim-1" (hereinafter also referred to as "Pim-1") means a polypeptide comprising the amino acid sequence of SEQ ID No: 1, and having serine/threonine kinase activity. Pim-1 was identified in T cell lymphomas caused by murine leukemia virus (MuLV) as a gene that is activated by MuLV insertion (Cuypers, H.T., Selten, G., Quint, W., Zijlstra, M., Maandag, E.R., Boelens, W., van Wezenbeek, P., Melief, C., Berns, A. "Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region." Cell, 37:141-150, 1984; and Selten, G., Cuypers, H.T. & Berns, A. "Proviral activation of the putative oncogene Pim-1 in MuLV induced T-cell lymphomas." EMBO J, 4:1793-1798, 1985 ~~Comerford, K.M., Wallace, T.J., Karhausen, J., Louis N.A., Montalto, M.C., Colgan, S.P. "Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene." Cancer Res., 62:3387-3394, 2001; and Niizeki, H., Kobayashi, M.,~~

~~Horiuchi, I., Akakura, N., Chen, J., Wang, J., Hamada, J., Seth, P., Katoh, H., Watanabe, H., Raz, A., Hosokawa, M. "Hypoxia enhances the expression of autocrine motility factor and the motility of human pancreatic cancer cells." Br. J Cancer, 86:1914-1919, 2002~~).

Please amend the paragraph bridging pages 10 and 11 as follows:

The polynucleotides encoding Pim-1 used in the present invention can be any polynucleotides, as long as they comprise a nucleotide sequence encoding Pim-1. DNAs are preferred, and the DNAs may be genomic DNAs, genomic DNA libraries, cDNAs derived from the cells or tissues described above, cDNA libraries derived from the cells or tissues described above, or synthetic DNAs, or such. The vectors used for the libraries may be bacteriophages, plasmids, cosmids, phagemids, or such. In addition, the DNAs can be directly amplified by reverse transcriptase polymerase chain reaction (hereinafter abbreviated as "RT-PCR") using total RNA or mRNA fractions prepared from the cells or tissues described above. For example, the DNAs encoding Pim-1 used in the present invention may be any DNAs, as long as the DNAs comprise the nucleotide sequence of SEQ ID No: 2, or comprise a nucleotide sequence that hybridizes under highly stringent conditions with a DNA comprising the nucleotide sequence of SEQ ID No: 2, and the DNAs encode a protein with characteristics that are substantially the same as the aforementioned protein comprising the amino acid sequence <u>encoded by the nucleotide sequence</u> of SEQ ID No: 2.

Please amend the text on page 19, line 16 through page 20, line 13 as follows:

The polypeptides comprising the amino acid sequence of SEQ ID No: 3 include, for example, proteins comprising an amino acid sequence with one or two or more (for example, 1 to 50 or so, or preferably 1 to 30 or so) amino acid deletions in the amino acid sequence of SEQ ID No: 3; proteins comprising an amino acid sequence with one or two or more (for example, 1 to 100 or so, or preferably 1 to 30 or so) amino acid additions in the amino acid sequence of SEQ ID No: <u>3</u> [[1]]; proteins comprising an amino acid sequence with one or two or more (for example, 1 to 100 or so, or preferably 1 to 30 or so) amino acid insertions in the amino acid sequence of SEQ ID No: <u>3</u> [[1]]; peptides comprising an amino acid sequence with one or two or more (for example, 1 to 100 or so, or preferably 1 to 30 or so) amino acid substitutions in the

amino acid sequence of SEQ ID No: 3 [[1]]; or proteins comprising an amino acid sequence with combinations of the above-mentioned alterations. The positions of these amino acid insertions, substitutions, and deletions are not particularly limited.

The C-terminus of proteins comprising the amino acid sequence of SEQ ID No: 3 may be a carboxyl group (-COOH), a carboxylate group ($-COO^-$), an amide group ($-CONH_2$), or an ester group (-COOR). R in the ester group includes, for example, an alkyl group of 1 to 6 carbon atoms, such as a methyl group, an ethyl group, an *n*-propyl group, an isopropyl group, or an *n*-butyl group; a cycloalkyl group of 3 to 8 carbon atoms such as a cyclopentyl group or a cyclohexyl group; an aryl group of 6 to 12 carbon atoms such as a phenyl group or an α-naphthyl group; a phenyl-alkyl group such as a benzyl group or a phenethyl group; an α-naphthyl-alkyl group such as an α-naphthylmethyl group; an aralkyl group of 7 to 14 carbon atoms; and a pivaloyloxymethyl group. When a protein represented by SEQ ID No: 3 [[1]] has carboxyl groups (or carboxylate groups) besides the one at the C terminus, those carboxyl groups may be amidated or esterified. These esters include, for example, the esters described above for the C terminus. Furthermore, the proteins represented by SEQ ID No: 3 may be the following: proteins in which the amino group of the N-terminal amino acid residue (for example, the methionine residue) is protected by a protecting group (such as an acyl group of 1 to 6 carbon atoms, such as an alkanoyl group of 1 to 6 carbon atoms including a formyl group or an acetyl group); proteins in which the N-terminal glutamine residue, which is produced by cleavage *in vivo*, is converted to a pyroglutamate; or proteins in which the substituents on the side chains of amino acids in the molecule (-OH, -SH, an amino group, an imidazole group, an indole group, a guanidinio group, or such) are protected with appropriate protecting groups (for example, an acyl group of 1 to 6 carbon atoms, such as an alkanoyl group of 1 to 6 carbon atoms including a formyl group or an acetyl group); or conjugated proteins such as sugar-chain-linked so-called glycoproteins.

Please amend the paragraph on page 21, lines 9-12 as follows:

The DNAs that encode the polypeptides comprising the amino acid sequence of SEQ ID No: 3, such as recombinant vectors carrying the polynucleotides comprising the nucleotide sequence of SEQ ID No: 4, can be produced by ligating the polynucleotide fragments

downstream of promoters in appropriate expression vectors. The above-mentioned vectors and promoters are used.

Please amend the paragraph on page 24, lines 6-11 as follows:

The above-mentioned therapeutic and preventive agents for cancer, and the anticancer agent potentiators, target cancers such as pancreatic cancer, esophageal cancer, gastric cancer, liver cancer, biliary tract cancer, spleen cancer, renal cancer, bladder cancer, uterine cancer, ovarian cancer, testicular cancer, thyroid cancer, ~~pancreatic cancer,~~ brain tumor, and blood tumor, and these agents are particularly effective against solid cancers with a reduced oxygen concentration in the cells.

Please amend the paragraph bridging pages 24 and 25 as follows:

The sensitivity of solid cancer cells to anticancer agents under low and normal oxygen partial pressures was investigated. Three types of solid pancreatic cancer cell lines (PCI-35 cells, KMP-4 cells, and PCI-43 cells) were used as the cells. For each type of cell, 2x 10^3 cells were cultured for six hours in the presence of 50 μg/mL of cisplatin under low oxygen partial pressure (1% oxygen, 5% carbondioxide; hereinafter, the same values in the Examples) or normal oxygen partial pressure (20% oxygen, 5% carbondioxide; hereinafter, the same values in the Examples). After culturing, the cells were washed twice with physiological phosphate buffer (pH7.4) to prepare samples. Dulbecco's ~~Dulceeco's~~ Modified Eagle's Medium/F12 was used as the medium for culturing (hereinafter, unless particularly specified, use of the same medium is assumed).

Please amend the paragraph on page 25, lines 5-13 as follows:

The results of FACS analysis are shown in Fig. 1. In Fig. 1, "Normoxia" means under normal oxygen partial pressure, and "Hypoxia ~~Hyposia~~" means under low oxygen partial pressure (the same hereinafter). The FACS analyses in the Examples can be described as staining of cells with PI and anti-anexin V, and sorting of cells based on the intensity of staining to show distributions such as those in Fig. 1. In the figures, the lower right quadrants represent early apoptotic cells, and the upper right quadrants represent late apoptotic cells. The lower left

quadrants represent live cells. The proportion of apoptotic cells, which is the total of the lower right and upper right quadrants, is shown as a percentage. Hereinafter, the FACS analyses of are shown in the same manner.

Please amend page 27, lines 20-26 as follows:

Expression of Pim-1 protein when various ~~pancreatic~~ cancer cells were exposed to hypoxia was observed over time. The cells used were HCT116, PCI-10, and PCI-43. Cells were sampled before culturing the cells, and after exposure to hypoxia for two and four hours. The cells were treated in the same manner as in Example 5, and were subjected to Western blotting. The results are shown in Fig. 6. In Fig. 6, β-actin was used as the detection control. As shown in Fig. 6, in all of the three types of cells used, the amount of Pim-1 was found to increase with time after exposure to hypoxia.

Please amend the paragraph on page 28, lines 7-16 as follows:

PCI-43 cells were cultured under normoxia in the presence of proteasome inhibitor ALLN. The cells were cultured for 16 [[six]] hours, sampled, and after immunoprecipitation of ubiquitin, were analyzed by Western blotting. The same procedure was performed as in Example 5, except that anti-ubiquitin antibody and anti-Pim-1 antibody were used as primary antibodies. The concentrations of ALLN were 0, 50, and 100 μM. The results are shown in Fig. 8. In Fig. 8, the left-hand side shows the results of using anti-Pim-1 antibody as the primary antibody, and the right-hand side shows the results of using anti-ubiquitin antibody as the primary antibody. As Fig. 8 shows, since the samples reacted with anti-Pim-1 antibody, Pim-1 is bound to the immunoprecipitated ubiquitin. More specifically, Pim-1 is first ubiquitinated, and the proteasome uses ubiquitin as a marker, and degrades Pim-1.

Please amend the paragraph on page 28, lines 25-29 as follows:

The cDNA of the dominant-negative Pim-1 lacking the kinase activity domain was amplified from the RT product of mRNA purified from PCI-10 cells, and was cloned into pCR4-TOPO. The cDNA ~~plasmid~~ was sequenced using an ABI377 automated sequencer (Applied Biosystems), and a DyeDeoxy Terminator kit (Perkin-Elmer). The cloned cDNA fragment was

then ligated into plasmid vector pcDNA3.1 (Invitrogen). The RT-PCR method is simply described below.

Please amend the paragraph on page 30, lines 22-24 as follows:

From mice used in Example 12 [[11]], tumor tissues ~~cells~~ were removed six days after the subcutaneous injection, and the tissues ~~cells~~ were subjected to immunohistochemical staining: PCNA, and TUNEL staining of apoptotic cells. The results are shown in Fig. 13.

Please replace the Sequence Listing after the drawings with the attached Sequence Listing.